United States Department of Agriculture
Office of the Chief Economist

Office of Risk Assessment and Cost-Benefit Analysis

TO: David Reeves, Director USDA/APHIS Pre-Clearance Programs
DATE: September 20, 2002
FROM: Mark Powell, USDA/ORACBA
RE: Revised Quantitative Analysis of Available Data on the Efficacy of Cold Treatment against Mediterranean Fruit Fly Larvae

Recent interceptions of live Mediterranean fruit fly (*Ceratitis capitata*) larvae in fruit that had been cold-treated during transit from abroad have led to a re-evaluation of the scientific basis for current USDA/APHIS Cold Treatment Schedule T107. Attached is a quantitative analysis of available scientific data regarding the efficacy of cold treatments to limit the risk of introducing Mediterranean fruit flies. A time-temperature response surface model based on the original experimental data was developed and evaluated based on subsequent experimental trials and recent surveillance data collected from shipping operations. The resultant model is reasonably robust and supports the conclusion by a panel of USDA scientists that the current treatment schedule falls short of the intended probit 9 level of security. Given the vintage of the data, methodological inconsistencies among studies, and the potential consequences of new introductions, additional research is warranted regarding the efficacy of low temperature - short duration treatments. Quantitative analysis of the currently available data suggests that future studies regarding the efficacy of cold storage should focus on low temperature - short duration treatments, where uncertainty about performance appears greatest. The analysis of the data from subsequent experiments and surveillance also demonstrates that for cold treatment trials most often resulting in zero survivors, appropriate statistical methods may be applied to a series of replicated trials of more manageable size. This offers a feasible alternative to conducting impracticably large mega-trials.

The original analysis was contained in my memo to you of July 5, 2002. The current analysis has been revised to reflect comments and incorporate new data received in response to the proposed Spanish clementine rule (67 FR 45922-45933). Please note, however, that the analysis has not yet been subject to independent peer review.

cc: Jim Schaub, USDA/ORACBA
Ron Sequeira, USDA/APHIS/PPQ
Matt Rhoads, USDA/APHIS/PPD

Quantitative Analysis of Available Data on the Efficacy of Cold Treatment against Mediterranean Fruit Fly Larvae

Introduction

In response to recent interceptions of live Mediterranean fruit fly (*Ceratitis capitata*) larvae in clementines that had been cold-treated during transit from Spain, a panel of United States Department of Agriculture (USDA) scientists and regulatory personnel was convened to review the available scientific literature and observations to date regarding the efficacy of APHIS fruit cold treatment schedule T107. Based on their review, the panel concluded that the existing T107-a treatment schedule falls short of the intended probit 9 level of phytosanitary security. (Cold treatment schedules T107-a, -c, and -f are authorized by USDA for control of *C. capitata*. The required cold treatment is commodity- and country-specific. The probit 9 security level corresponds to a 3.2×10^{-5} probability of survival.) The panel therefore recommended increasing the length of cold treatment currently required at each temperature by two days (Table 1). The panel also recommended that USDA establish research plans to verify the proposed new cold treatment parameters (APHIS 2002a).

Table 1. Present T107-a vs. Proposed Cold Treatment Schedule

	Days	
Temperature °F (°C)	T107-a	APHIS (2002a)
32 (0.0)	10	12
33 (0.6)	11	13
34 (1.1)	12	14
35 (1.6)	14	16
36 (2.2)	16	18

The purpose of this quantitative analysis is to develop and evaluate a response surface model relating Mediterranean fruit fly (*Ceratitis capitata*) larval survival to cold treatment time-temperature combinations based on available data. (Unlike a basic linear regression model with a single predictor variable, a response surface model can be plotted in three dimensions, indicating the response of the dependent variable (e.g., *C. capitata* survival) as two input variables (e.g., time and temperature) are varied.) A simple model was developed on the basis of multiple logistic regression analysis of larval survival data reported by Back and Pemberton (1916), the original research which informed development of the present T107 treatment schedule (APHIS 2002a). The predictions of

the response surface model are then compared to results of subsequent *C. capitata* larvae cold treatment trials conducted under the relevant time-temperature treatment combinations (Nel 1936, Sproul 1976, Hill et al. 1988, Jessup et al. 1993, Santaballa et al. 1999, and De Lima et al. 2002) as well as surveillance data collected from marine shipping vessels in 2001 (APHIS 2002b). The analysis is not intended to elaborate the definitive model of *C. capitata* larval response to cold treatment, rather it primarily aims to corroborate whether the existing cold treatment schedule fails to achieve the intended level of protection, assess broad trends in the available data, and provide input regarding the focus of future data acquisition.

Data and Methods

Response Surface Model Development. Due to the coverage of time-temperature combinations spanning the entire range of concern to phytosanitary programs and the reporting of unsummarized results, the best currently available data for development of a response surface model to estimate *C. capitata* larval survival under cold treatment is Back and Pemberton (1916). Apples, peaches, and kamani nuts were used as *C. capitata* hosts. Six cold-storage temperature levels were included in the analysis, with the temperature data converted to Celsius and coded as the midpoint in the case of nominal storage temperature intervals: 32 °F (0 °C), 32-33 °F (0.28 °C), 33-34°F (0.83 °C), 34-36°F (1.67 °C), 36°F (2.22 °C), and 36-40°F (3.33 °C). (The final storage temperature level was included to inform the high temperature and long duration regions of the response surface. Data on exposures at the 38-40 °F and 40-45 °F storage temperature levels reported by Back and Pemberton (1916) were excluded to limit the effect of independent variable measurement error on the multiple regression analysis.) The duration of cold storage varied by storage temperature, with a minimum of 15 d and a maximum of 30 d.

Although Back and Pemberton (1916) did not completely report their methodology, it appears that the duration of treatment refers to cold storage time, instead of the cold treatment time elapsed once the fruit cooled to a given temperature. This presents a potentially significant source of measurement error and an inconsistency with more recent studies and the present T107 cold treatment requirements. Mason and McBride (1934) reported that the time required for the interior of fruits (apples, oranges, and avocados) to reach storage room temperatures of 28 to 31°F ranged from 18 to 48 h. In general, precooling time depends on the volume and packing of fruit being treated. It is also conceivable that larval survival under cold treatment depends on the cooling rate

(i.e., a biological response may differ if stress is applied gradually or swiftly). Studies also differ in the survival measurement endpoint. Back and Pemberton (1916) measured larval survival based on observation 24-48 h after removal from cold storage. Although the detection of live larvae may be judged sufficient for phytosanitary inspection purposes, some subsequent studies recorded survivors only as those that emerged from the fruit and attained the pupal stage because some larvae that appear alive after cold storage fail to pupate (Mason and McBride 1934). Furthermore, in infested fruits that are not subject to cold treatment, survival of pupae to the adult stage may vary from 70-80 percent (Santaballa et al. 1999). Consequently, the response surface model based on the Back and Pemberton (1916) cold storage data is hypothesized to demarcate a plausible upper-bound on effective larval survival under compliant cold treatment.

Back and Pemberton (1916) identified the observed larval stage as 1st, 2nd, or 3rd instars, and statistical analysis tends to support the conclusion that later instars are somewhat more cold-tolerant (see discussion below). Some subsequent studies failed to distinguish larval stage, however. For each time-temperature combination, therefore, Back and Pemberton (1916) data on the number of 1st, 2nd, and 3rd instars found alive and dead after cold treatment were combined for the purposes of response surface model development.

The time-temperature response surface was obtained using a standard logistic regression procedure (SAS© PROC LOGISTIC), and assuming a simple main effects model:

$$\text{logit}(p_s) = \ln\left(\frac{p_s}{1-p_s}\right) = b_0 + b_1 * \text{temperature}(^{\circ}C) + b_2 * \text{time}(days), \quad \text{(eq.1)}$$

$$\text{where: est.prob.survival}(\hat{p}_s) = \frac{\exp(\text{logit}(p_s))}{1+\exp(\text{logit}(p_s))}.$$

Under this model, the logit link function is assumed to transform the underlying model into a linear function of the parameters (eq. 1), and the error about the fit regression curve is assumed to be binomially distributed (Brown and Rothery 1993). In developing the response surface model, three generalized linear model link functions were considered: the logit, normit, and complementary log-log. Each was fit with and without a logarithmic transformation of time and temperature. The logit is the inverse of the cumulative logistic distribution function. Like the normal distribution, it is symmetric about the mean, but the logistic is a more heavy-tailed distribution. The normit distribution is the inverse of the cumulative standard normal distribution function. The

more familiar term probit is often used, although conventionally the probit function contains the additive constant 5 to avoid negative values. (Thus, the probit 9 level refers to the area under the standard normal distribution beyond 4 standard deviations above the mean, or simply 3.2×10^{-5}.) Applying both a logarithmic transformation and the probit link function assumes the tolerances to be lognormally distributed within the population. The complementary log-log function is the inverse of the cumulative extreme-value function (also called the Gompertz distribution), which is skewed. These empirical models represent a range of model forms, but they do not imply a specific mechanism of larval mortality due to low temperature, which is not well understood at this time. Among the models considered, the model based on the untransformed data and the logit link function (eq. 1) was selected on the basis of goodness-of-fit criteria. Using a more flexible empirical model with additional terms would improve the statistical goodness-of-fit, but the candidate models were selected on the basis of parsimony and ease of interpretation.

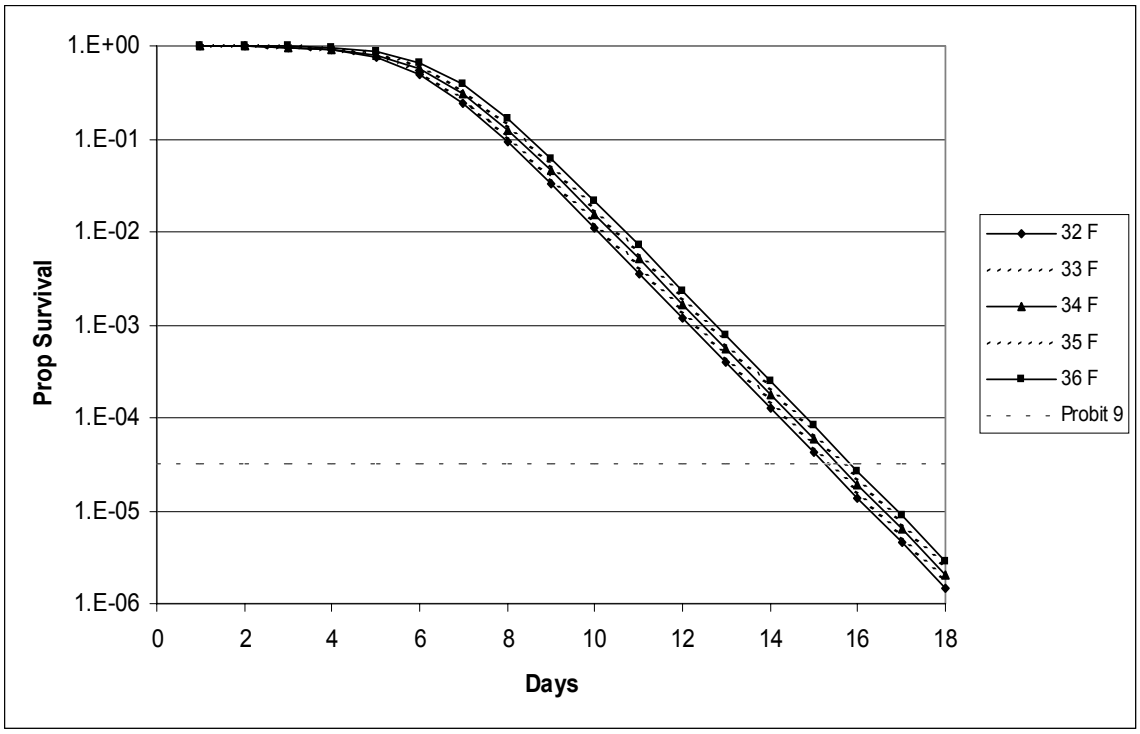
Regression Analysis Results

The multiple logistic regression analysis results presented in Table 2 indicate that the maximum likelihood estimates (MLE) of the model parameters are statistically significant ($p<0.01$). The different variance estimates for the model parameters (depending on whether the error is assumed to be binomially distributed or is estimated empirically, i.e by dividing the deviance goodness of fit statistic by its degrees of freedom) do not affect the parameter estimates but indicate extra-binomial dispersion. The response surface model is presented in Figure 1. Although temperature was found to be statistically significant, the model suggests that within the range of cold storage conditions considered, one additional day of cold storage may yield substantially more protection than lowering the storage temperature by 1 °F (0.56 °C).

Table 2. Logistic Regression Results

		Std Error		P > Chi-Sq
Parameter	MLE	Binomial Dispersion	Empirical Dispersion	(Empirical Dispersion)
b_0 - Intercept	6.6448	0.0884	0.5234	< 0.0001
b_1 – Temp (°C)	0.3063	0.0199	0.1177	0.0093
b_2 – Days	-1.1155	0.0127	0.0753	< 0.0001

Figure 1. Response Surface Model for *C. capitata* Larval Survival under Cold Storage



For cold treatment periods of less than 16 d, the results tend to support the finding that the existing T107-a treatment schedule achieves less than the probit 9 level of phytosanitary security ($p_s = 3.2 \times 10^{-5}$), as well as the conclusion that the duration of cold treatment would need to be increased if this level of security is to be achieved (APHIS 2002a).

Model Evaluation

In order to assess the robustness of the model based on the Back and Pemberton (1916) data, model predictions were compared with 95% confidence intervals constructed about the results of *C. capitata* larvae cold treatment trials conducted under similar time-temperature combinations, as well as recent surveillance of shipping operations. As indicated above, however, some subsequent studies failed to identify insect stage. Therefore the model was first evaluated regarding sensitivity to the effect of insect stage.

Multiple logistic regression analysis of the Back and Pemberton (1916) data with a model including categorical variables for insect stage:

$$\text{logit}(p_s) = f(\textit{temperature},\ \textit{time},\ \textit{stage}) \quad (\text{eq. } 2)$$

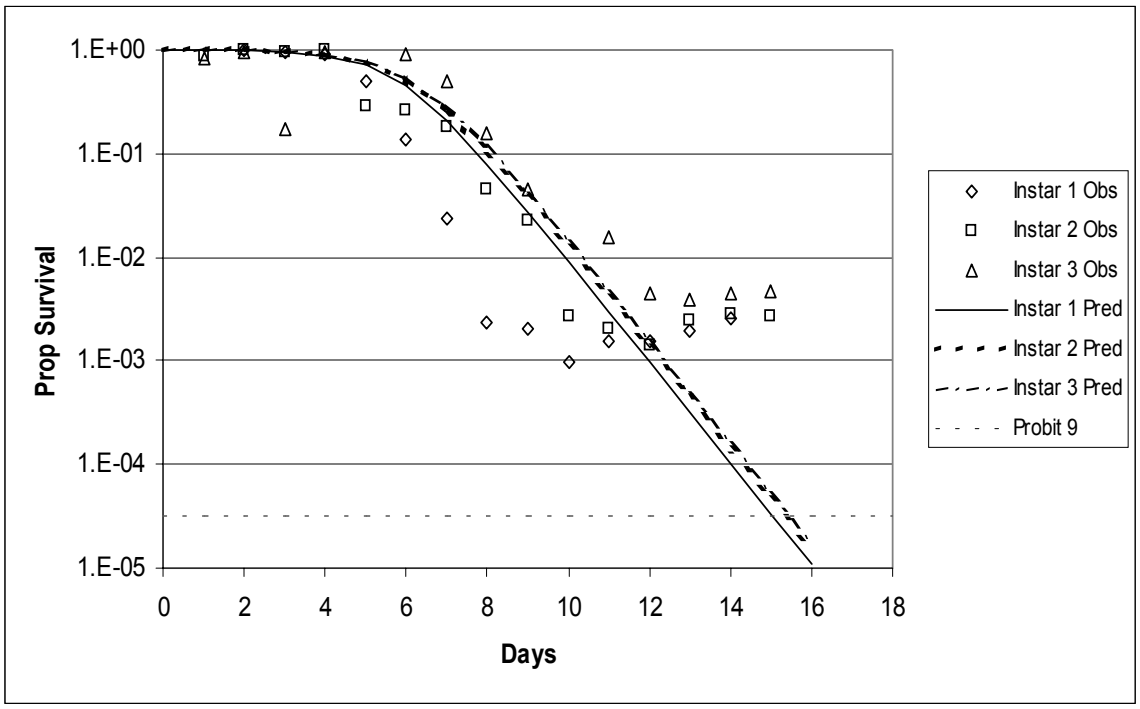
indicates that eggs were less likely to survive a given time-temperature combination than larvae. Also, 1st and 2nd instars were less likely to survive a given time-temperature combination than 3rd instars (Table 3). (In comparing the probability of a binary outcome for group A vs. group B, an odds ratio of 1.0 indicates that there is no association between the independent variable and the dependent variable. If the odds ratio is less than 1.0, group A is less likely to have the outcome than group B.) In the comparisons presented in Table 3, note that the 95% confidence intervals about the odds ratios do not contain 1.0. (Due to a large proportion of incomplete cases, the eggs v. larvae comparison presented in Table 3 was conducted separately from the instar comparisons. In an analysis of the reduced set of complete cases containing data on all four stages, only the eggs vs. 3rd instar comparison (yielding an odds ratio of 0.66) was statistically significant ($p<0.05$).)

Table 3. Survival Odds Ratio for *C. capitata* Stages

Comparison	Odds Ratio	95% Confidence Interval
Eggs vs. Larvae	0.617	0.572-0.666
Instar 1 vs. 3	0.633	0.564-0.711
Instar 2 vs. 3	0.873	0.782-0.975

Despite the statistically significant odds ratio comparisons, the magnitude of the effect of larval stage on the cold treatment time estimated to achieve a probit 9 level of security appears insubstantial. Figure 2, for example, overlays the Back and Pemberton (1916) instar-specific data reported for cold treatment at 32 °F (0 °C) with the corresponding model predictions. (The statistical treatment of trials with zero survivors presented in Figure 2 is described below.) Similarly, Jessup et al. (1993) and Santaballa et al. (1999) reported overlap among instars in the 95% confidence intervals for the time required to achieve a given level of mortality.

Figure 2. *C. capitata* Instar Survival at 32 °F



Various immature stages of fruit fly may be present in a given commercial consignment of fruit (Sproul 1976, Santaballa et al. 1999). In order to be reliable, therefore, cold treatments must be designed for the most cold-tolerant stage of *C. capitata*. Despite the apparent lack of sensitivity of modeled results to instar stage, in cases where subsequent studies distinguished among observed larval stages, the predictions of the response surface model were compared to confidence intervals for reports specific to more mature or cold-tolerant larvae. (In some studies, 2nd instars were judged to be most cold tolerant.) Table 4 presents the data used to evaluate the time-temperature response surface model.

Table 4. Data Used to Evaluate Response Surface Model

Study	Temp	Days	No. Larvae Treated (n_i)	Survivors (s)
Nel (1936)	1.1 °C (34°F)	9	1,670	50
	1.1 °C (34°F)	10	1,140	26
	1.1 °C (34°F)	11	2,905	3
	1.1 °C (34°F)	12	2,902	0
	1.1 °C (34°F)	12	3,907	0
Sproul (1976)	0.5 °C (32.9°F)	14	22,000	0
	0.5 °C (32.9°F)	14	800	0
	0.5 °C (32.9°F)	14	10,100	0
	0.5 °C (32.9°F)	14	12,900	0
	0.5 °C (32.9°F)	14	13,700	0
	1.5 °C (34.7°F)	16	8,000	0
	1.5 °C (34.7°F)	16	12,000	0
	1.5 °C (34.7°F)	16	21,800	0
	1.5 °C (34.7°F)	16	13,200	0
Hill et al. (1988)	1 °C (33.8°F)	16	18,904	0
	1 °C (33.8°F)	16	11,668	0
	1 °C (33.8°F)	16	10,584	0
	1.5 °C (34.7°F)	16	41,099	3
Jessup et al. (1993)	1 °C (33.8°F)	14	10,010	0
	1 °C (33.8°F)	14	10,140	0
	1 °C (33.8°F)	14	10,080	0
	1 °C (33.8°F)	14	20,015	0
	1 °C (33.8°F)	14	13,158	0
	1 °C (33.8°F)	14	10,170	0
Santaballa et al. (1999)	2 °C (35.6°F)	10	935	10
	2 °C (35.6°F)	12	935	5
	2 °C (35.6°F)	14	935	0
	2 °C (35.6°F)	16	11,317	0
	2 °C (35.6°F)	16	10,295	0
	2 °C (35.6°F)	16	10,376	0
De Lima et al. (2002)	2 °C (35.6°F)	16	141,441	0
	2 °C (35.6°F)	16	165,894	0
	2 °C (35.6°F)	16	133,788	0
	2 °C (35.6°F)	16	108,732	0
	2 °C (35.6°F)	18	132,216	0
APHIS (2002b)	0 °C (32°F)*	10*	212	2

*assumed

The fruits, *C. capitata* larval stages and strains, and survival measurement endpoints used differ among studies. Nel (1936) used nectarines, peaches, plums, and grapes as host material and used live larvae as the survival measurement endpoint. The *C. capitata* larval stage was unidentified. Sproul (1976) used Granny Smith apples, and the survival measurement endpoint was emergent pupae. The data presented are for old (primarily 3rd instar) *C. capitata* larvae. Hill et al. (1988) used Valencia and Navel oranges, and the survival measurement endpoint was emergent pupae. The data presented are for old (primarily 3rd instar) *C. capitata* larvae. Jessup et al. (1993) used Lisbon and Eureka lemons, and the survival measurement endpoint was emergent pupae. The data presented are for 2nd instars, which were found to be most cold tolerant. Santaballa et al. (1999) used clementines, and the survival measurement endpoint was live larvae. The data presented for trials conducted for up to 14 d are for old (primarily 3rd instar) larvae; young (1st and 2nd instar) larvae were used for the 16 d-trials. (In the former, smaller trials, no statistically significant differences in cold tolerance were observed between young and old larvae.) De Lima et al. (2002) used Lisbon lemons for 16 d trials and Navel and Valencia oranges and Ellendale and Murcott tangors for 18 d trials. The survival measurement endpoint was emergent pupae, and the data presented are for 2nd instar larvae. Finally, in surveillance conducted by the USDA Animal and Plant Health Inspection Service (APHIS) during 2001, *C. capitata* larvae were detected in 29 of 20,460 clementines that were cut and inspected after cold-treatment on 80 shipping vessels. A total of 212 larvae were detected, 2 live and 210 dead (APHIS 2002b). Larval stage was unidentified. Cold treatments likely varied among the shipping vessels, but a treatment of 0 °C (32°F) for 10 d is assumed since it is the shortest T107-a-compliant treatment, and due to the perishable nature of the commodity. (The minimum marine shipping vessel transit time between Spain and the New York metropolitan area, for example, is approximately 7 d (http://www.shipguide.com). As indicated below, evaluation of the model with respect to the surveillance data is robust to departures from the assumed treatment time and temperature.) While not subject to experimental controls, the 2001 surveillance data arguably represents the best available evidence regarding the current operational performance of cold treatment.

Confidence intervals constructed about the experimental trial and surveillance results were obtained assuming that the probability of larval survival for a given time-temperature combination is beta distributed. Because the beta distribution is the conjugate prior for the proportion of successes (p_s) when values (s) from a sample (n) follow a binomial distribution, the beta distribution is used to characterize the uncertainty about proportions arising from binomial processes (Vose 2000). To estimate the beta

distribution parameters for trials with some surviving *C. capitata* larvae, the method of matching moments was used (Evans et al. 1993):

$$\text{If } s \sim \text{binomial}(n, p_s), \qquad \text{(eq. 3)}$$
$$\text{where: } \mathrm{p}(s = \mathrm{x}) = C_x^n p^x (1-p)^{n-x},$$
$$\text{then } \mathrm{p_s} \sim \text{beta}(\alpha, \beta),$$
$$\text{where:}$$
$$\hat{\alpha} = \bar{x}\{[\bar{x}(1-\bar{x})/s.d.^2] - 1\},$$
$$\hat{\beta} = (1-\bar{x})\{[\bar{x}(1-\bar{x})/s.d.^2] - 1,$$
$$\bar{x} = \frac{\text{survivors}(s)}{\text{no. larvae treated}(n)},$$
$$s.d.^2 = \bar{x}(1-\bar{x})/(n-1).$$

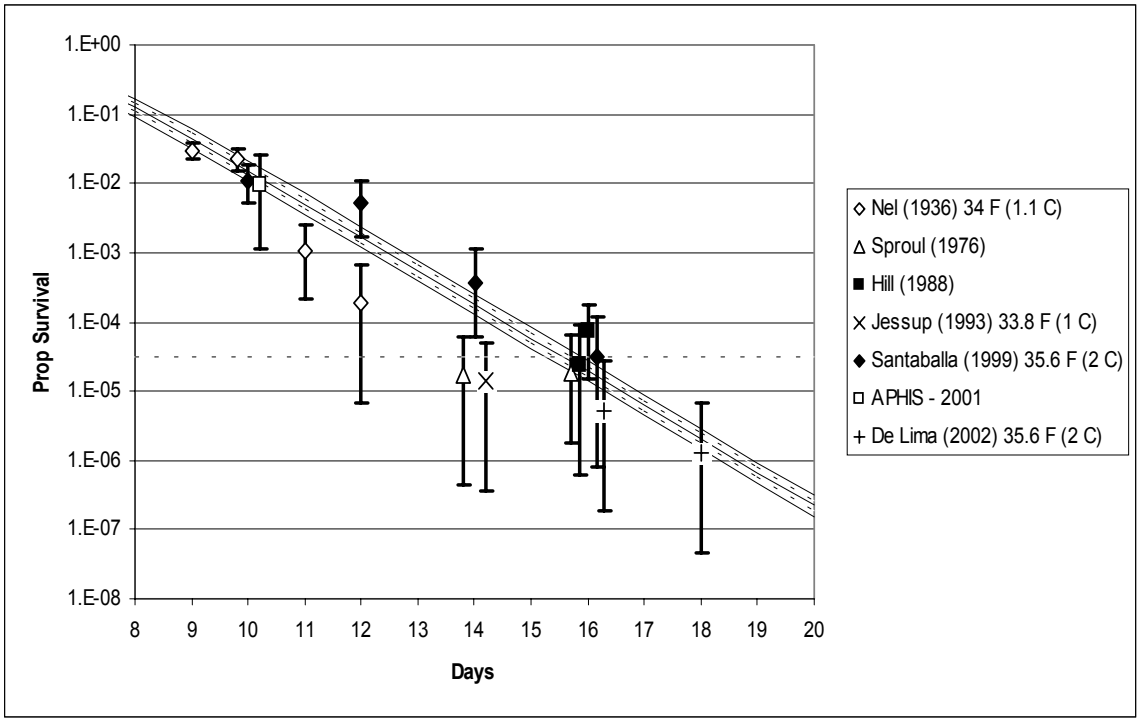
For trials in which no larvae survived cold treatment, sample moments cannot be obtained. (Estimating the beta distribution parameters would involve dividing by zero, since the sample variance (s.d.^{2}) is zero.) For these trials, Bayesian statistical methods were used to estimate the beta distribution parameters (Vose 2000). Bayes Rule implies:

$$\text{posterior prob.}(p_s|s,n) \propto \text{prior prob.}(p_s) * \text{lik}(s,n|p_s), \quad \text{(eq. 4)}$$
$$\text{where: lik}(s = 0, n = n_i|p_s) = (1\text{-}p_s)^{n_i}.$$

Note that the likelihood of observing zero out of n_i survivors, given p_s, follows from the binomial distribution where s = 0 (eq. 3). The Bayesian estimation operation proceeded in sequential fashion, beginning with a maximally uncertain prior distribution (uniform(0,1)), evaluating the likelihood of observing the results of the first trial (s=0, n_1) for discretized p_s values (from 1 x 10^{-7} to 1 x 10^{-3} at increments of 1x10^{-7}), and obtaining a posterior uncertainty distribution by calculating the product of the initial prior and the likelihood of having observed the data over the values of p_s. The resultant posterior uncertainty distribution then becomes the prior distribution for the second trial (s=0, n_2), and so on. The process is repeated until all of the trials from a given study with zero survivors for a particular time-temperature combination have been evaluated. The moments of the final posterior distribution can then be calculated to obtain beta distribution parameter estimates (eq. 3). Given estimated beta distribution parameter values, 95% confidence intervals for each of the relevant time-temperature combinations

were constructed by obtaining the 2.5th and 97.5th percentiles of the distribution (using the Microsoft© Excel™ BETAINV function).
Figure 3 presents the confidence intervals constructed about the evaluation data overlaid with the response surface model based on the Back and Pemberton (1916) data.

Figure 3. Evaluation of Response Surface Model for *C. capitata* Larval Survival under Cold Storage



Despite the use of a simple empirically-based model and the fact that the data are more than 80 years old, the model appears reasonably robust, particularly as an upper-bound on *C. capitata* larval survival under cold treatment. Four observations from two subsequent studies (Hill et al. 1988, Santaballa et al. 1999) indicate a mean survival proportion above the predicted response, but the confidence limits contain the model prediction in each case. Note also the consistency between the APHIS 2001 surveillance data and the model prediction. Although the parameters of the cold treatments performed on the shipping vessels were assumed to be 32 °F (0 °C) for 10 d, the confidence interval about the observed proportion of larval survival overlaps with the model predictions assuming that the cold treatments varied from 32°F (0 °C) for 9 d to 36 °F (2.2 °C) for 12.5 d.

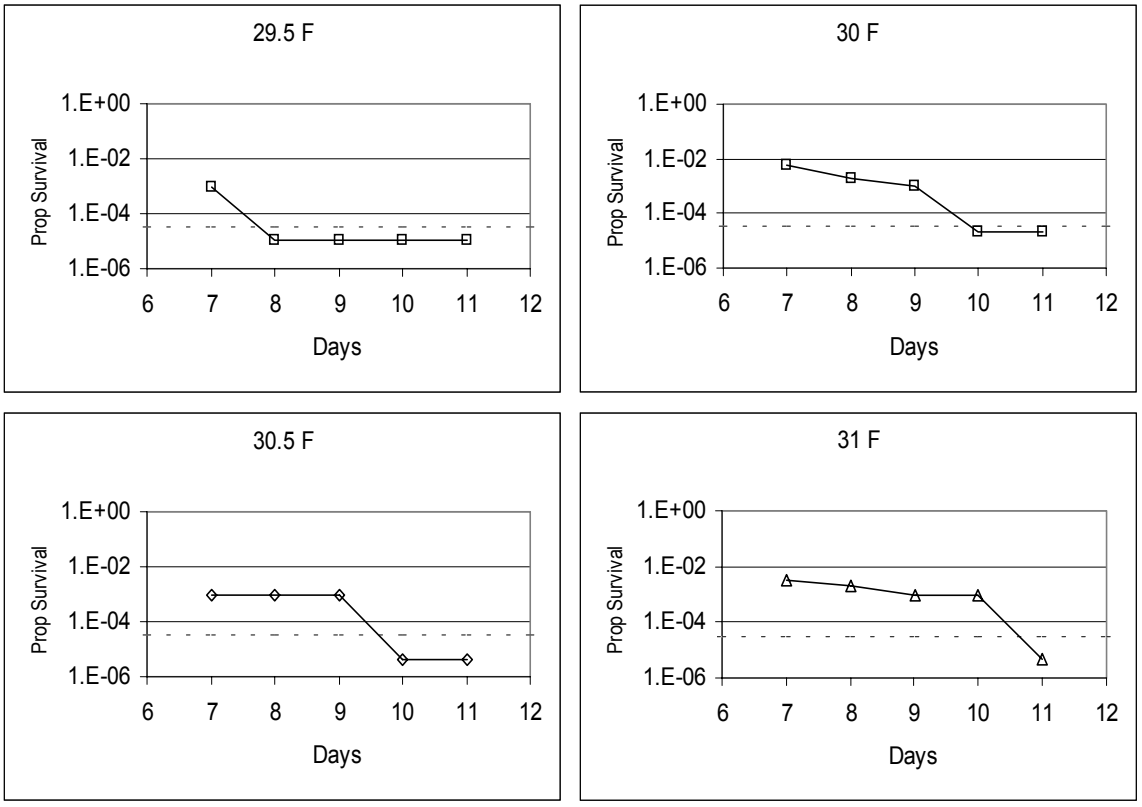
In general, the evaluation data analysis and the model predictions are particularly consistent in the low temperature – short duration region of the response surface, and both lines of evidence suggest that existing cold treatment requirements fall short of achieving the probit 9 level of security (3.2×10^{-5}). Specifically, the confidence interval about the 34 °F (1.1 °C), 12 d trial reported by Nel (1936) departs significantly from the response surface model prediction, but both indicate a low level of confidence that the current T107-a combination of 34 °F (1.1 °C), 12 d achieves the probit 9 level of security. In contrast, the confidence interval about the trial reported by Jessup et al. (1993) for 33.8 °F (1 °C), 14 d suggests a high degree of confidence that the proposed 34 °F (1.1 ° C), 14 d treatment (APHIS 2002a) should achieve the probit 9 level of security. The surface response model predictions and the confidence intervals about three of the 16 d trials (Sproul (1976) at 34.7 °F (1.5 °C), Santaballa et al. (1999) at 35.6 °F (2 °C), and De Lima et al. (2002) at 35.6 °F (2 °C)) suggest a degree of confidence that the proposed 35 °F (1.6 °C), 16 d treatment (APHIS 2002a) should achieve the probit 9 level of security. Similarly, the surface response model predictions and the confidence interval about the 35.6 °F (2 °C), 18 d trial reported by De Lima et al. (2002) indicate a high degree of confidence that the proposed 36°F (2.2 °C), 18 d treatment (APHIS 2002a) should achieve the probit 9 level of protection.

While these results suggest that the proposed 36°F (2.2 °C), 18 d treatment may be more than sufficient to attain the probit 9 level of security, note that the confidence interval about the 34.7 °F (1.5 °C), 16 d trial reported by Hill et al. (1988) indicates a low level of confidence of achieving the probit 9 level. This serves as a reminder that some variance in cold treatment performance can be expected depending on cold treatment procedures, study materials and methods, and other factors. Note, however, the confidence intervals for the 16-day trials conducted at the same temperatures but with different fruits (Sproul (1976) and Hill et al. (1988) at 34.7 °F (1.5 °C); Santaballa et al. (1999) and De Lima et al. (2002) at 35.6 °F (2 °C)). Here, the confidence intervals span multiple orders of magnitude, while the point estimates differ by less than an order or magnitude. This suggests that at very low levels of larval survival (ca. probit 9), the uncertainty associated with the response for a specific time-temperature-fruit combination may be greater than the variability in response due to different fruit hosts, at least for the cultivars considered.

Uncertainty remains regarding what statistical model form best describes the observed cold treatment data. The response surface model predictions and the confidence intervals overlap at 10 and 16-18 d, but there is some disagreement at intermediate treatment durations, indicating that the model fails to account for all of the observed variation.

Similarly, the regression analysis of the Back and Pemberton (1916) also indicates substantial overdisperion about the response surface model (Table 2), suggesting that alternatives to the continuously sloping logistic model should be considered for the purpose of improved response surface modeling. The biological mechanism of larval mortality due to low temperature is not well understood, but if a critical physiological point exists (e.g., beyond which cell walls rapidly lose integrity), this might suggest using a discontinuous (e.g., splined) model form. Empirical evidence suggesting that an alternative, biologically-based model might be considered is provided by the results of trials conducted by Mason and McBride (1934) on a combination of both *C. capitata* eggs and larvae, suggesting a discontinuous pattern of response to cold treatment (Figure 4).

Figure 4. Cold Treatment of *C. capitata* eggs and Larvae at 29.5-31 °F (-1.4 to - 0.6 °C)



Discussion

Although the original work was conducted more than 80 years ago, the conclusions derived from the response surface model appear reasonably robust in comparison to more recent studies and surveillance data. Overall, the quantitative analysis suggests that within the range considered (32-36 °F (0-2.2 ° C)), the duration of cold treatment may be more important than the nominal storage temperature in driving *C. capitata* larval survival to very low levels. The currently available cold treatment data, however, are sparse and primitive, for the most part. Given the vintage of some of the data, methodological inconsistencies, and consequences of new introductions, additional research is warranted, especially to verify the efficacy of low temperature - short duration treatments.

While the broad coverage of the Back and Pemberton (1916) data makes it best suited for constructing a time-temperature response surface model for *C. capita* larval survival, for the purposes of revising the regulatory cold treatment schedule, elaborating a complete response surface and refining its fit are unnecessary. Instead, the efficacy of discrete time-temperature combinations may be evaluated independently. The evaluation data were analyzed assuming only that the uncertainty regarding the proportion of survivors can be characterized by the beta distribution, i.e. larval morality results from a binomial process with unknown but invariant p_s. Focusing on a limited set of discrete time-temperature combinations permits us to relax or simply avoid the far more numerous statistical assumptions inherent to response surface methods (e.g., the assumption that the logit transformation (eq. 1) is linearizing). This is of particular concern because predictions at the extremely low survival levels relevant to phytosanitary programs may be dominated not by the observed data but by the assumed statistical model form (e.g., heavy-tailed or light-tailed distribution). Given that more recent studies have illuminated some of the discrete time-temperature cold treatment combinations of primary concern, the greatest remaining uncertainty appears to be whether treatments of less than 14 days at temperatures in the 32-33 °F (0.0 – 0.6 °C) range will achieve the probit 9 level of security. The analysis suggests, therefore, that the efficacy of low temperature - short duration treatments should be a primary focus of new data acquisition.

The practical significance of the Bayesian statistical methods used to analyze the evaluation data is that researchers need not conduct unfeasibly large trials to assess the performance of cold treatments resulting in very low levels of insect survival. Some may believe that only trials with a very large number of treated insects will permit statistical analysis of probit 9 security levels. Consider, however, that for a trial with zero observed

survivors to provide 95% confidence that probit 9 level security has been achieved, the number of treated insects would have to be approximately 92,500 (The 95th percentile of a beta(1, 92501) distribution = 3.2 x 10^{-5}. See eq. 5 below for this parameterization of the beta distribution.) The belief that such mega-trials are necessary can pose a strong deterrent to initiating needed research.

While the "absence of evidence is not evidence of absence," the number of treated insects need not be impracticably large to permit informative analysis. The Bayesian statistical methods described above demonstrate that trials with zero survivors *can* provide useful information about the true underlying probability of survival at a given time-temperature combination. In such cases, it is more than intuitive that our confidence that the probability of survival is small increases with sample size. Therefore, replicated trials of more manageable size offer a feasible alternative to an impracticably large mega-trial. Conveniently, as the cumulative sample size becomes large (e.g., $n=\Sigma n_i > 10{,}000$), the statistical estimation process can be greatly simplified by noting that the beta distribution parameter estimates resulting from the laborious Bayesian operation closely approximate those obtained by assuming a uniform(0,1) prior (implying that in the absence of information, all values of p_s between 0 and 1 are considered equally likely) and a single, large trial (Vose 2000):

$$\hat{\alpha} = s + 1, \quad (\text{eq. } 5)$$
$$\hat{\beta} = n - s + 1.$$

A legitimate concern arising from the use of Bayesian statistical methods is that the prior distribution is subjectively defined. In cases where data are sparse, the Bayesian prior distribution may dominate the observed data in determining the resultant posterior distribution. As data accumulate, however, the influence of the prior distribution diminishes, and the empirical data come to statistically dominate the posterior distribution (Robert and Casella 1999).

Entirely separate from the question of whether the cold treatment attains the intended level of mortality is whether the probit 9 level of security is either necessary or sufficient to maintain an acceptably low risk of establishment of new *C. capitata* populations outside the pest's current distribution. Given a large enough volume of infested fruit imports, even the probit 9 level of security could be overwhelmed. One the other hand, attaining a greater level of security via treatment alone may be impracticable. Cold treatment, however, is not the only hurdle to clear. There are multiple sources of

resistance to establishment of a new colony, which depends on pre-treatment infestation levels, dynamics of escaping mortality and predation in a novel ecological community, synchronous emergence of adult male and female survivors, density-dependent probability of encountering a mate, spatially and temporally specific likelihood of encountering a suitable host for oviposition, and other factors.

Disclaimers: Reference herein to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government.

References

APHIS (USDA Animal and Plant Health Inspection Service). 2002a. Evaluation of cold storage treatment against Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera:Tephritidae). Oxford, NC, Oxford Plant Protection Center.

APHIS (USDA Animal and Plant Health Inspection Service). 2002b. Risk mitigation for Mediterranean fruit flies with special emphasis on risk reduction for commercial imports of clementines (several varieties of *Citrus reticulata*) from Spain. Raleigh, NC, Center for Plant Health Science and Technology.

Back, E. A. and C. E. Pemberton. 1916. Effect of cold-storage temperatures upon the Mediterranean fruit fly. Journal of Agricultural Research 5:657-666.

Brown, D. and P. Rothery. 1993a. Models in Biology: Mathematics, Statistics and Computing. John Wiley & Sons, NY.

De Lima, C. P. F., A. Jessup, and R. McLauchlan. 2002. Cold disinfestation of citrus using different temperature x time combinations. South Perth, Horticulture Australia.

Evans, M., N. Hastings, and B. Peacock. 1993. Statistical Distributions, 2nd ed. John Wiley & Sons, Inc., NY.

Hill, A. R., C. J. Rigney, and A. N. Sproul. 1988. Cold storage of oranges as disinfestation treatment against the fruit flies *Dacus tyroni* (Froggatt) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Journal of Economic Entomology 81:257-260.

Jessup, A. J., C. P. F. De Lima, C. W. Hood, R. F. Sloggett, A. M. Harris, and M. Beckingham. 1993. Quarantine disinfestation of lemons against *Bactrocera tyroni* and *Ceratitis capitata* (Diptera: Tephritidae) using cold storage. Journal of Economic Entomology 86:798-802.

Mason, A. C. and O. C. McBride. 1934. Effect of low temperatures on the Mediterranean fruit fly in infested fruit. Journal of Economic Entomology 27:897-902.

Nel, R. G. 1936. The utilization of low temperatures in the sterilization of deciduous fruit infested with the immature stages of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Pretoria, South Africa, Union of South Africa Department of Agriculture

and Forestry.

Robert, C. P. and G. Casella. 1999. Monte Carlo Statistical Methods. Springer, NY.

Santaballa, E., R. Laborda, and M. Cerda. 1999. Informe sobre tratamiento frigorifico de cuarentena contra *Ceratitis capitata* (Wied) para exportar mandarinas clementinas a Japon. Valencia, Spain, Universidad Politecnica de Valencia.

Sproul, A. N. 1976. Disinfestation of Western Australian Granny Smith apples by cold treatment against the egg and larval stages of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Australian Journal of Experimental Agriculture and Animal Husbandry 16:280-285.

Vose, D. 2000. Risk Analysis: A Quantitative Guide, 2nd ed. John Wiley & Sons., Ltd, Chichester, England.